

Reproductive Diapause in *Speyeria* (Lepidoptera: Nymphalidae)¹

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Abstract. A delay in ovarian development (reproductive diapause) occurs in adult females of *Speyeria coronis* (Behr) and *Speyeria zerene* (Boisduval) from California during the warm-dry weather of the summer flight period. Females copulate soon after emergence; males show little evidence for a delay in spermatogenesis. Termination of the female diapause is hastened by injection of juvenile hormone and by exposure to short-day photoperiods. Diapause delays the onset of oviposition until later summer or early fall and thus decreases the exposure time of overwintering first instar larvae to desiccating conditions.

Introduction

Speyeria Scudder is a North American genus of Nymphalid butterflies characterized by their univoltinism, exclusive use of violets (*Viola* sp.) as host plants, and overwintering as diapausing first instar larvae. There are at least 14 closely related species, 10 of which occur in California (Brittnacher et al., 1978).

Edwards (1874, 1897) found that females of several species from the Eastern US, *S. cybele* (Fabr.), *S. diana* (Cramer), and *S. aphrodite* (Fabr.), mate immediately after emerging from May through July but often do not become reproductively mature and commence oviposition until August or September. These observations strongly suggest a reproductive diapause (Beck, 1980; Masaki, 1980) but no subsequent investigations have been made on the Eastern *Speyeria*. While studying the genetic relationships (Brittnacher et al., 1978) and reproductive biology of Western *Speyeria*, I observed that *S. coronis* (Behr) and *S. zerene* (Boisduval) from California display a similar prereproductive delay. In this paper I document the reproductive diapause of *S. coronis* and *S. zerene* and present results of laboratory studies on the environmental and endocrine bases for diapause termination.

Materials and Methods

Females of *S. zerene* were obtained from the following California localities during the summers of 1973-1975: Boggs Mountain State Forest nr. Cobb, 850 m, Lake

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Co. (BM); Mt. Ingalls, 2500 m, Plumas Co. (MI); Fales Hot Springs, 1.5 mi. W Devils Gate Pass, 2300 m, Mono Co. (FH), and Yuba Pass, 2000 m, Sierra Co. (YP). *S. coronis* females were collected at (MI) and (YP) during the same years.

The subspecies collected and locations are as follows: *S. zerene zerene* (Boisduval) (BM), *S. zerene conchyliatus* (Comstock) (MI, YP), *S. zerene malcolmi* (Comstock) (FH), and *S. coronis snyderi* (Skinner) (MI, YP). The peak flight times, and availability of fresh females, of the high elevation Sierran *S. zerene* populations occurred approximately one month after the Coast Range (BM) population (July 1 vs. August 1).

A total of 211 butterflies was used in this study. Collections were made using a standard aerial insect net. Live individuals were placed into glassine envelopes in the field and returned to the laboratory under refrigeration. Only insects in freshly emerged condition, based upon wing wear and scale loss, were used in the experiments. Live weights were determined on a sample of *S. zerene* females collected in 1975 at the BM locality.

The reproductive status of freshly emerged females of *S. zerene* from BM, FH, MI, and YP and *S. coronis* from MI was determined by dissecting abdomens in 70% ETOH. Ovarioles were inspected for oocyte development and mature oocytes (oocytes with a ridged chorion) were counted and recorded. The condition and general magnitude of the fat body was observed but not quantified.

To determine the length of the preoviposition period, females of *S. zerene* (BM, YP) and *S. coronis* (YP) were individually maintained in one-liter cardboard containers. Leaves of *Viola papilionacea* Pursh served as the oviposition substrate. To maintain leaf succulence, cut ends of leaf petioles were inserted through a hole in the bottom of each container into a jar of water. Adults were fed once daily on a 10% honey-water solution using a holding device described earlier (Sims, 1979), and maintained under either a LD 15:9 or a LD 12:12 photoperiod cycle at 24 ± 1 C within Percival environmental chambers. At the 38°N latitude encompassing the study populations, the chosen photoperiods approximately represent the natural day length from June 15 - July 15 and from September 15 - October 1.

The time interval between initial exposure to violet leaves and date of first oviposition was used as a descriptor of reproductive diapause length in *S. coronis* and *S. zerene*. This seemed reasonable since females of both these and other *Speyeria* species that are reproductively mature (i.e. those with mature oocytes) show no additional behavioral delay of oviposition within the containers used here (Sims, unpubl. data).

An endocrine basis of reproductive development in *S. zerene* (BM) was studied using juvenile hormone III (JH III) obtained from Zoecon Corp., Palo Alto, CA. A 5.0 ul capacity microsyringe was used to inject 32 females with 3 ug JH III in 3 ul of pure olive oil carrier. Olive oil alone was injected into 28 controls from the same population. Injection was made along the abdominal pleurite area on the day following capture.

To examine the effect of day length on reproductive diapause, the experimental (JH III + olive oil) and control (olive oil) groups were divided with half the females in each being maintained at LD 15:9 and LD 12:12, 24 ± 1 C. The oviposition response of 5 additional non-injected *S. zerene* (BM) females was determined at LD 12:12. Maintenance of females and egg monitoring was performed as previously described.

Results

The reproductive condition of freshly emerged females from one *S. coronis* and four *S. zerene* populations is shown in Table 1. Only *S. zerene* from the eastern Sierra Nevada (FH) showed signs of reproductive maturity such as mature oocytes and partial depletion of the fat body. All other populations of both species lacked visible oocyte development while fat body completely filled the abdominal cavity. All females had mated at least once as indicated by the presence of a spermatophore in the bursa copulatrix. Three *S. zerene* (2 FH, 1 MI) had mated twice and contained two spermatophores. One of the 3 *S. coronis* and 85/113 (75%) of the *S. zerene* had hardened mating plugs of accessory gland material typically extending to the ostium bursae.

S. coronis (YP) females had a significantly longer preoviposition period at LD 15:9 than either of the two *S. zerene* populations (BM, FH) (Table 2). *S. zerene* females from the eastern Sierra Nevada (FH) had a shorter preoviposition period than the central Coast Range (BM) population. This finding corresponds to the relatively advanced state of reproductive maturity found among young FH *S. zerene* females (Table 1).

The data indicate that relatively short photoperiods decrease the length of the preoviposition period (Table 2). When the statistically homogeneous (injected controls and non-injected) values for individuals at LD 15:9 and LD 12:12 respectively are combined and compared between photoperiods, the difference between means (LD 15:9 = 23.00 ± 1.63 days; LD 12:12 = 17.78 ± 1.13) is significant ($t = 2.52$, 40 df, $p < 0.02$).

The mean live weight of *S. zerene* females studied from BM was 0.44 ± 0.10 (1 SD) g ($n = 46$). The insects were therefore injected with a dosage of JH approximately equal to 7 ug per g of body weight. This dosage is similar to that used to study reproductive diapause in other butterfly species (Pan and Wyatt, 1971; Herman and Dallman, 1981). Injection of

Table 1. Reproductive status of freshly emerged females of *S. coronis* and *S. zerene* from California.

Species	Population	Collection date (1973)	Sample size	Mean number of mature ova in ovarioles (± 1 SE)
<i>S. coronis</i>	(MI)	8 July	3	0
<i>S. zerene</i>	(BM)	4 July	31	0
<i>S. zerene</i>	(MI)	8 July	40	0
<i>S. zerene</i>	(YP)	26 July	10	0
<i>S. zerene</i>	(FH)	7 August	32	25.6 ± 5.3

Table 2. Preoviposition periods of *Speyeria coronis* and *Speyeria zerene*.

Species (population)	Treatment (photoperiod- JH injection)	Sample size	Mean number of days to first oviposition ($\pm 1SE$) ^b
<i>S. coronis</i> (YP)	LD 15:9 No injection	9	36.00 \pm 3.27 a
<i>S. zerene</i> (BM)	LD 15:9 No injection	13	25.23 \pm 2.35 b
<i>S. zerene</i> (BM)	LD 12:12 No injection	5	20.20 \pm 2.31 bc
<i>S. zerene</i> (BM)	LD 15:9 - Control ^a	10	20.10 \pm 1.91 bc
<i>S. zerene</i> (BM)	LD 12:12 - Control	14	16.92 \pm 1.26 c
<i>S. zerene</i> (BM)	LD 12:12 - JH	16	12.37 \pm 1.27 d
<i>S. zerene</i> (BM)	LD 15:9 - JH	10	11.20 \pm 1.37 d
<i>S. zerene</i> (FH)	LD 15:9 No injection	8	6.25 \pm 0.86 e

^aControls injected with olive oil carrier alone.

^bMeans followed by the same letter are not significantly different at 0.05 level (Duncan's Multiple Range Test).

juvenile hormone significantly reduced the preoviposition period of *S. zerene* at both LD 15:9 and LD 12:12.

Discussion

Reproductive dormancy of the estival type has been documented in the Lepidoptera groups Satyrinae and Noctuidae (Jacobson, 1960; Scali, 1971; Edwards, 1973; Weissman, 1972) but appears to be unrecorded among the Nymphalinae. Delayed ovarian development with estivation is one method for avoiding hot, dry, and often host plant-deficient summer conditions which could decrease larval survivorship. Small (1-2 mm) first instar diapausing larvae of *S. coronis* and *S. zerene* are particularly susceptible to death from desiccation despite their use of protected overwintering sites in the soil or within plant material and replenishment of lost body fluids by drinking water (Sims, unpubl.). Populations of *S. coronis* and *S. zerene* are characteristically found in xeric habitats of the Sonoran and Transition Plant Life Zones and eggs are laid on or near violets in these areas (Hammond, 1981). Reproductive diapause in *S. coronis*, *S. zerene* and perhaps other *Speyeria* species may have evolved as a means to reduce the exposure time of first instar larvae to the withering conditions of summer. This contention is supported by evidence for interpopulation variability of adult diapause intensity in *S. zerene*. Diapause

variability appears to correspond to the duration of summer and desiccation stress within the larval habitat as well as the emergence and flight time of adults. The FH population, for example, which flies about one month later, at a higher elevation, and in cooler and moister summer climate than the BM population, shows a weaker diapause response.

The mode of diapause induction is unknown. Initiation of larval development is synchronized with the growth of new violet foliage in early spring and proceeds under fluctuating warm-cool temperatures and increasing day lengths. Adults emerge under long-day warm conditions.

Photoperiod appears to be important in diapause termination and initiation of oogenesis. Short days facilitate diapause cessation and act as a reliable environmental signal for diapause termination. Day length and perhaps other unstudied factors may exert their diapause-terminating effect(s) by reactivating the corpora allata which produce JH. The response to JH found here is similar to that displayed by other Nymphalids with hibernal diapause (Endo, 1970; Benz, 1972; Herman and Bennett, 1975; Herman and Dallmann, 1981). A more detailed comparison of the similarities between the endocrine bases of estival and hibernal diapause in Nymphalids, however, remains to be done.

Males emerge slightly before females at the start of the flight season in each population. An uneven sex ratio is thus established beginning with a preponderance of males and ending with mostly females. Using wing wear as an approximation of insect age, both males and females appear to be sexually receptive soon after emergence. All fresh condition females observed had already mated and three *S. zerene* (BM) males collected in copula were also in fresh condition.

At least a 3 to 5 week long female reproductive dormancy is likely under field conditions during July and August. These are the warmest and driest months in the *Speyeria* habitat of cismontane California (Dale, 1966). The behavior of females during this time is unknown but may differ between the two species. *S. coronis*, for example, is highly vagile and is often seen miles from suitable oviposition sites (Hammond, 1974). Diapause in *S. coronis* may represent an oogenesis-flight syndrome (Johnson, 1969) in which dispersing individuals are reproductively inactive. *S. zerene* generally seems to be less vagile but late season (Sept.-Oct.) captures of females away from breeding sites in California suggest considerable inter-intrapopulation variability in adult movement (Mattoon, pers. comm.). A comparison of reproductive diapause between the Western and Eastern *Speyeria* species would help to clarify the evolutionary basis for this adaptation.

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